Acta Biomaterialia 9 (2013) 8690–8703

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

A layer-by-layer approach to natural polymer-derived bioactive coatings on magnesium alloys ☆

Sangeetha Kunjukunju [a], Abhijit Roy [a], Madhumati Ramanathan [a], Boeun Lee [a], Joe E. Candiello [a], Prashant N. Kumta [a,b,c,d,e,*]

[a] Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA 15261, USA
[b] Department of Chemical and Petroleum Engineering, University of Pittsburgh, Pittsburgh, PA 15261, USA
[c] Mechanical Engineering and Materials Science, University of Pittsburgh, Pittsburgh, PA 15261, USA
[d] Center for Craniofacial Regeneration, University of Pittsburgh, Pittsburgh, PA 15261, USA
[e] Center for Complex Engineered Multifunctional Materials (CCEMM), University of Pittsburgh, Pittsburgh, PA 15261, USA

ARTICLE INFO

Article history:
Received 7 November 2012
Received in revised form 13 May 2013
Accepted 14 May 2013
Available online 22 May 2013

Keywords:
Magnesium
Coating
Polyelectrolytes
Layer by layer
Biodegradation
Corrosion

ABSTRACT

The development of polyelectrolyte multilayered coatings on magnesium alloy substrates that can be used for controlled delivery of growth factors and required biomolecules from the surface of these degradable implants could have a significant impact in the field of bone tissue regeneration. The current work reports on the fabrication of multilayered coatings of alginate and poly-L-lysine on alkaline- and fluoride-pretreated AZ31 substrates using a layer-by-layer (LbL) technique under physiological conditions. Furthermore, these coatings were surface functionalized by chemical cross-linking and fibronectin immobilization, and the resultant changes in surface properties have been shown to influence the cellular activity of these multilayered films. The physicochemical characteristics of these coated substrates have been investigated using attenuated total reflectance Fourier transform infrared spectroscopy, atomic force microscopy, scanning electron microscopy and energy-dispersive X-ray spectroscopy. Cytocompatibility studies using MC3T3-E1 osteoblasts show that the fluoride-pretreated, cross-linked and fibronectin-immobilized LbL-coated substrates are more bioactive and less cytotoxic than the hydroxide-pretreated, cross-linked and fibronectin-immobilized LbL-coated samples. The in vitro degradation results show that the multilayered coatings of these natural polysaccharide- and synthetic polyamino acid-based polyelectrolytes do not alter the degradation kinetics of the substrates; however, the pretreatment conditions have a significant impact on the overall coating degradation behavior. These preliminary results collectively show the potential use of LbL coatings on magnesium-based degradable scaffolds to improve their surface bioactivity.

1. Introduction

Recently, Mg and Mg alloys have attracted much interest as a new class of biodegradable metals for cardiovascular and orthopedic applications due to their low density (1.74 g cc^{-1}), greater fracture toughness, and a good match of the elastic modulus and compressive yield strengths to natural bone [1–6]. Degradable Mg is considered to be biocompatible, non-toxic and also osteoconductive [4,7–11]. However, Mg-based alloys are known to be highly reactive in the electrochemical series, undergoing rapid electrochemical dissolution in body fluid, resulting in the immediate release of copious volumes of hydrogen that are unacceptable for human body tissue [1–6]. Additionally, rapid degradation of Mg alloys may cause an adverse tissue reaction due to the transient high local concentration of released Mg^{2+} and other ions [7,12,13]. The rapid degradation and limited bioactivity of Mg implant in vivo have prevented its widespread clinical application. Recent advances in controlling the purity and use of selective alloying elements along with different alloy processing parameters have resulted in significant reduction in corrosion rate [4,6,11,14]. Although these alloying and processing techniques may improve the biocorrosion resistance of magnesium alloys to a great extent, it is unlikely that these techniques could significantly alter the cell and tissue response, especially in the early stages after implantation [15,16]. It has been well established that the final success and lifetime of dental and orthopedic implants are determined by the quality of the bone–implant reaction, which is characterized by a tight bond between the bone and the implant surface without

☆ Part of the Biodegradable Metals Conference 2012 Special Issue, edited by Professor Frank Witte and Professor Diego Mantovani.

* Corresponding author at: Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA 15261, USA. Tel.: +1 (412) 648 0223.
E-mail address: pkumta@pitt.edu (P.N. Kumta).

1742-7061/$ - see front matter © 2013 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2013.05.013

the occurrence of an intervening fibrous tissue layer. The rate, quantity and quality of bone response depends not only on the intrinsic properties of the implanted biomaterials, but also largely on the surface chemistry. The time frame for the establishment of direct bone-to-implant contact and the mechanical nature of the connection between the bone and the implant surface are influenced by the nature of the implant surface itself. Therefore, distinctive alterations of implant surfaces may lead to different and unique chemical as well as physical surface properties that might potentially lead to changes in the bone-to-implant interactions [15]. Thus, tailoring of the magnesium-based biomaterial's surface with a suitable coating may be of immense importance in increasing its biocompatibility and osteoconductivity. Moreover, these unique coatings could also help to prevent the rapid corrosion of the surfaces.

In the last few years, several researchers have attempted different coating methods to deposit corrosion-protective and bioactive layers of magnesium fluoride, calcium phosphate and biodegradable polymers on different Mg alloys [16–20]. In addition to corrosion protection and bioactivity, other factors need to be addressed for coatings developed on degradable magnesium-based implants. For example, the coating must degrade with time and the degradation rate preferably should be faster than that of the Mg-based scaffold itself. Furthermore, if necessary, the coating should temporarily protect the underlying scaffolds from degradation in vivo, and should also be able to deliver drugs and different growth factors in a controlled predesigned manner. Considering all these aspects, polymeric coatings appear especially interesting because of the diversity of the chemical and physical properties they offer. Due to the infinite variability of polymers, chemical composition and structures, they have been prepared with a wide range of properties and functions.

In addition to this advancement in the functionality of the polymers, there has been enormous progress in the techniques used to (self-)assemble these polymers into functional materials. Among the different assembling strategies, the layer-by-layer (LbL) approach has attracted considerable attention, especially for biological applications [21–23], since it exploits the principle of electrostatic attractive forces between oppositely charged building blocks – thereby making it a versatile, simple method for the incorporation of synthetic and natural biocompatible and biodegradable polyelectrolytes into biomedical coatings [21–28]. Polysaccharides, amino acids and proteins such as chitosan [29], hyaluronic acid [26], alginate [30,31], poly-L-lysine [32] and collagen [33] are examples of natural polyelectrolytes that have been used by researchers for fabricating multilayers. The conditions under which LbLs are built up (pH, rinsing solution, ionic strength, number of layering steps, order of assembly and chemical cross-linking) dictate the morphology, thickness and internal structure of the coatings, which in turn determine their distinctive bioactivities. It has been shown that different parameters, such as hydrophobicity and hydrophilicity, surface charge, roughness and free energy of LbL films, affect protein adsorption and subsequently cellular adhesion [21–23].

Many previous studies have reported that films can alter the activities of cells through their mechanical properties, especially stiffness. Cross-linking is a common technique that has been used to improve the biofunctionality of various polyelectrolyte films by modulating their mechanical properties [24,34,35]. Due to the very close interrelations between mechanical properties and biological activities, it is of interest to determine the effect of cross-linking on surface properties as well as biological activities. Moreover, multilayer LbL coatings can be further functionalized in a controlled fashion such that the functionalized surface can interact with desired proteins, peptides, enzymes and drugs [22,27]. Controlled immobilization of different molecules on the coating surface not only enhances the bioactivity and biocompatibility of these coatings, but also promotes rapid regeneration of desired tissues.

Despite the advantages offered by LbL, few efforts have been made so far to use this versatile technique to develop corrosion-protective as well as bioactive coatings on Mg alloys. For example, Cai et al. [36] developed multilayered coatings on AZ91D substrates using polyethyleneimine (PEI), polystyrene sulfonate and 8-hydroxyquinoline. They reported that these multilayered coatings improved the corrosion resistance of AZ91D substrates and were also cytocompatible. During the course of the present study, Liu et al. [37] reported on the use of the LbL self-assembly technique combined with micro-arc oxidation (MAO) to improve the corrosion resistance of WE43 magnesium alloy substrates. Although potentiodynamic polarization tests and hydrogen evolution measurements showed that the formed MAO/LbL coating significantly enhanced corrosion resistance of WE43 magnesium alloy in simulated body fluid, the biocompatibility of these coated surfaces is not known.

The present study reports on the LbL formation of biocompatible organic natural polymer-based coatings on the magnesium alloy, AZ31. Alkaline-treated AZ31 as well as magnesium-fluoride-pretreated AZ31 were used as substrates. Alginate and poly-L-lysine were chosen as the polyanion and polycation for the LbL assembly system, respectively. The physicochemical properties of the LbL and cross-linked LbL films were characterized using different analytical techniques and the cytocompatibility of these films were also tested. Furthermore, the effect of LbL coating and cross-linking of multilayer films on the corrosion, cytocompatibility and bioactivity of the AZ31 substrates were studied in detail.

2. Experimental procedure

2.1. Materials

AZ31. alloy was obtained from Alfa Aesar (Ward Hill, MA, USA). Poly-L-lysine hydrobromide (PLL, Mw 15–30 kDa), sodium alginate (ALG, Mw 80–120 kDa, $\geqslant$2,000 cP), PEI (branched, Mw 70–150 kDa), fibronectin and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma (St. Louis, MO, USA). 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide sodium salt (NHS) were obtained from Thermo Scientific Pierce. The substrates were cut into squares of 10 mm $\times$ 10 mm $\times$ 0.8 mm and cleaned using acid etching and repeated washing with acetone. The substrates were then polished with 320, 600 and finally 1200 grit SiC paper and cleaned under ultrasonication using acetone.

2.2. Substrate treatments

2.2.1. Treatment of AZ31 with NaOH

The polished substrates were soaked in 5 M NaOH solution at 60 °C for 2 h, then cleaned thoroughly with deionized water and dried at 60 °C. These hydroxide-treated AZ31 substrates are denoted by HAZ31 in the rest of this article.

2.2.2. Treatment of AZ31 with HF

MgF_2 coatings on AZ31 were obtained by immersing cleaned substrates in HF (48–51 wt%, Acros Organics) at room temperature for 24 h under constant stirring. The MgF_2-coated substrates (denoted by FAZ31) were first washed thoroughly with distilled water and then with acetone, and were finally dried in air at 50 °C for 20 min.

ID	Title	Pages
581	A layer-by-layer approach to natural polymer-derived bioactive coatings on magnesium alloys ☆	14